whereinone or more amino acid residues are substituted, deleted, inserted, and/or added, so long as the amino acid residues that are important for the adjuvant activity are retained. The mutant may include not only mutations in its amino acid sequence but also mutations in sugar residues, which are other constituents of the toxin.

Functional groups of amino acid residues are important for protein function. For example, serine residue and threonine residue containing an OH group, glutamic acid residue and aspartic acid residue containing a COOH group, and lysine residue and arginine residue containing an NH_2 group, often form the active center and play important roles in the expression of activity. Just as in the cases of many enzymes and functional proteins, it is believed that OH groups, COOH groups, NH_2 groups, and such in the amino acid residues forming the toxin molecule are closely associated with the expression of biological activities including, for example, enzyme activity, activity of enhancing immunity, activity of binding to a receptor, etc. Furthermore, the number of amino acids may be another index showing the importance of OH group. For example, the total contents of the three types of amino acids, namely serine, threonine, and tyrosine residue, each of which contains an OH group, in the polypeptides of cholera toxin, heat-labile toxin of human-type *E. coli*, and pertussis toxin are about 18%, 22% and 20%, respectively. The contents suggest the high possibility of their involvement in the active center. Accordingly, a recombinant mutant toxin that contains deletions or substitutions with other amino acids of these amino acids closely related to the active center may easily cause remarkable changes in its three-dimensional structure. Thus, it is highly possible that it loses not only toxic activity but also the activity of enhancing immunity simultaneously. As an example supporting this proposition, the present inventors have observed that in a recombinant mutant of *E. coli* heat-labile toxin, in which a lysine residue was substituted for the glutamic acid residue at amino acid position 112 from the N terminal end, while the toxic activity was reduced to about 1/2,860, the activity of enhancing the immunity was lost (K. Komase et al., Vaccine 16, 248-254, 1998).

Based on these findings, the present inventors selected three

amino acid residues, namely serine residue, glutamic acid residue, and lysine residue, from amino acid residues having the above-mentioned three types of functional groups as being important amino acid residues for adjuvant activity. The importance of these amino acid residues for the activities of a variety of toxins has been reported previously.

Previous reports on the change of toxic activity associated with the substitutions of these amino acid residues are summarized in Table 1. Those reports described in Table 1 as having negative activity are reports in which substantially no residual toxic activity was detected in the selected condition of measurement, but in which no quantitative comparison was carried out. Adjuvant activity was detedted in *E. coli* heat-labile mutant toxin but the activity of enhancing immunity was not sufficiently high. Further, 10 times as much as natural cholera toxin was required for a mutant cholera toxin to give the same level of activity of enhancing immunity as natural cholera toxin. The indication "and others" means that other amino acid residues in addition to the residues indicated were also substituted. As shown, glutamic acid residue and serine residue are important residues that are often targets to be substituted with other amino acid residues. However many reports describe that such substitutions frequently result in loss or declines of the adjuvant activity.

In the present invention, amino acid residues required for the maintenance of sufficient adjuvant activity includes, in addition to these important amino acid residues that are targets for substitution, lysine residue containing an NH_2 group, which is a representative of basic amino acids. Lysine residues play an important role in the attenuation of toxin. For example, it is believed that in the formalin treatment, formalin attacks the ϵNH_2 group of lysine, and the formation of a Schiff base at that site results in changes of three-dimensional structure, and thus the attenuation is achieved as a consequence. Accordingly, when lysine residues are retained, it is possible to readily attain a high degree of attenuation in which the toxicity is reduced to at least one-two thousandth that of the natural toxin.

Table 1

type of toxin	substitution of amino acid residues (reference)	alteration of toxic activity and adjuvant activity	
E. coli heat-labile toxin	position112 Glu→Lys(1)	Y: 1/2860	−
	position 63 Ser→Lys(2)	A: negative	+
cholera toxin	position 63 Ser→Lys(2)	A: negative	±
	position 61 Ser→Phe(3)	C: 1/1000000	++
	position 112 Glu→Lys(4)	C: 1/1000000	++
pertussis toxin	position 129 Glu→Gly and others(5)	Y: 1/1000000	−
diphtheria toxin	position 162 Glu→Lys and others(6)	A: negative	−

Marker of toxic activity A: ADP-ribosyltransferase activity
C: spindle-forming test with CHO cells
Y: Y-1 cell morphologic transformation test

Study reports
(1) Komase et al., Vaccine 16, 248-254, 1998
(2) G. Douce et al., Infec. Immun. 65, 2821-2828, 1997
(3) S. Yamamoto Proc. Natl. Acad. Sci. USA, 94, 5267-5272, 1997
(4) S. Yamamoto J. Exp. Med. 185, 1203-1210, 1997
(5) M. Roberts et al., Infec. Immun. 63, 2100-2108, 1995
(6) T. Uchida et al., J. Biol. Chem. 248, 3838-3844, 1973

However, a highly attenuated toxin, of which toxicity is reduced to at least one-two thousandth or, more preferably, at least one-ten thousandth that of the natural toxin and having a strong activity of enhancing immunity, is not predictable from the previous reports.

For example, the cholera A subunit is thought to be responsible for the major toxic effect of cholera toxin. It has been expounded that the toxic activity is expressed when the strong ADP-ribosyltransferase activity of A subunit disturbs the balance of intracellular level of cAMP concentration in the cell permeated by the toxin. The toxic activity of *E. coli* heat-labile toxin is also expressed by the same mechanism. A paper was published, wherein it